

Award Number:
W81XWH-11-1-0579

TITLE:
Creation of a mouse with stress-induced dystonia: control of an ATPase chaperone

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REPORT DATE:
April 2013

TYPE OF REPORT:
Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE April 2013		2. REPORT TYPE Final		3. DATES COVERED 30 September 2011 – 29 March 2013	
4. TITLE AND SUBTITLE Creation of a mouse with stress-induced dystonia: control of an ATPase chaperone				5a. CONTRACT NUMBER W81XWH-11-1-0579	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Kathleen J. Sweadner, PhD E-Mail: sweadner@helix.mgh.harvard.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Massachusetts General Hospital Boston, Massachusetts 02114-2554				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Dystonia is the 3 rd most common movement disorder, and some forms are brought on by stress. Investigators have not yet succeeded in making a genetic mouse model of dystonia by conventional approaches. A novel strategy was designed to combine three mutant mouse strains together by breeding to reduce the expression of a protein known to be mutated in an inherited human dystonia triggered by stress. There were problems with one of the mouse strains, however, which lost one of its genetic modifications, and a different strategy was adopted. This was successful, and a mouse with the desired dystonic symptoms was obtained. It has two mutations, one a dominantly inherited gene with 100% penetrance, and the other a strain-specific modifier gene that controls how strong the symptoms are. These genes were mapped to two loci on two different chromosomes. The behavior of the mouse was extensively characterized. Swim stress brought about very severe symptoms, but the mice fully recovered to a moderate dystonic baseline. As planned, electrophysiology and electromyography were performed, showing the co-contraction of opposing muscles, the hallmark of dystonia.					
15. SUBJECT TERMS Dystonia, genetically modified mice, stress, gene mutations, animal model of disease.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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Introduction

Dystonia is the 3rd most common motor disorder. Our understanding of the underlying mechanisms is still incomplete, and there are a variety of hypotheses that should be testable if there were a realistic animal model. Mice with mutations in genes known to cause dystonia in humans are so far virtually asymptomatic. Only mild motor deficiencies have been seen, such as slips while trying to walk an elevated beam. It is important to have a mouse model that actually exhibits the motor disorder for investigation of physiology and treatments. Please note that there are now two Statement of Works, the original one, and a modified one that reflects the changes we had to make because of technical obstacles. The second SOW was submitted at the time we requested a no-cost extension to complete the work. The original one called for step-wise breeding together of three strains of mice, each with a different genetic modification, to combine the modifications. Then we were to test their ability to produce symptoms of dystonia in response to stress, and that plan would take the full year. One of the critical mice proved to have lost a gene modification before it was sent to us, however, and our attempts to rebuild it were not successful. Fortunately though, we succeeded in producing a different dystonic mouse with features that are arguably even better than we had originally hoped. The second SOW was to increase the number of inbred mice for motor behavior testing; increase the number of hybrid mice for locus mapping; do the motor testing at baseline and after swim stress and alcohol stress; and complete the genetic analysis of the two genes that we determined are required to produce dystonia. Consequently this progress report covers about 6 months of our attempts to carry out the original aims, and 6 months of the very successful efforts to produce and characterize a stress-sensitive dystonic mouse.

Body

1. First 6 months, original SOW

The project started with the importation of mice with a floxed Atp1b1 gene, and their mating with mice with a knockout of Atp1a3. The first cycle of breeding was successful (3 months) and progeny with the apparently right combination of genotypes were obtained. The second generation of interbreeding was underway (2 months) when we learned from the lab that donated the floxed mice that they discovered that one of the three loxP sites was missing. This occurred before the mice were sent to us, apparently during a breeding bottleneck that happened when the collaborators moved their lab from California to Delaware. To be specific, one of the loxP sites was unstable and was lost presumably due to recombination with the wild type chromosome during normal meiosis. Our routine genetic tests were not designed to detect the loss of a loxP site, since that seldom happens. The method of detection was PCR based on primers in the modified DNA sequences, and did not happen to include the site that was lost. A recombination event like that, between two loxP sites and the third, is statistically improbable, and if the donating lab had kept a moderately large colony of mice, they would have had other mice from the colony to use. It is only because they reduced the size of their colony for the move and bad luck that only the bad recombinant was saved. We had to suspend the breeding.

The donating lab made their own attempt to reconstruct the mouse, but had bad luck with the commercial firm it was contracted to. They then shipped me the last precious vials of recombinant mouse ES cells, and the Mouse Genetics facility at our institution made two attempts to recover living mice. Unfortunately the cells were too old, and it was not successful.

Currently, the donating lab is starting over, and will make floxed Atp1b1 mice by two completely independent strategies. However, that will take about a year, and it will be too late for this grant. We will have to find new funding after viable mice have been produced.

The second task of the first SOW was to test the mice for dystonia, and obviously we did not get that far with the original plan. However, in the meantime we had discovered an

unrelated mouse with striking symptoms of dystonia, and when symptoms in the new mice were seen to be provoked by stress, it was clear that we could accomplish the original scientific and translational objectives and advance research in dystonia despite the setbacks.

2. Second 6 months, the dystonic mouse.

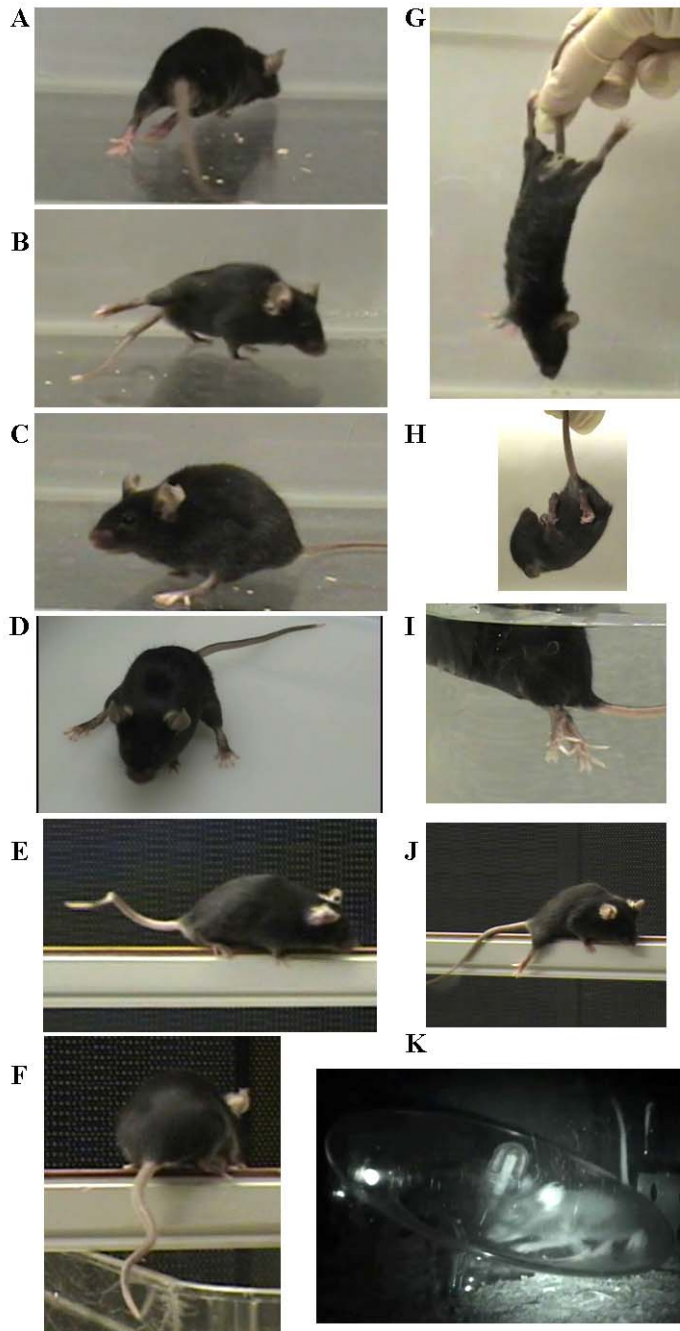


Figure legend:

Figure 1 shows the dystonic mouse line with its striking symptoms. The postures pictured in A-D are not held continuously, because the mice gain control when they are relaxed.

A. Hyperextension of the hindlimbs is typically seen in the home cage when the mice are disturbed: legs stiff, extended rearwards, palm up, toes spread.

B. In a somewhat more stressful novel environment, kicks are seen during random ambulation and turning.

C and D. Sitting posture. Although it looks ataxic (wide-based) it is apparently due to hyperextension.

E and F. Curly tail is caused by dystonic activation of lateral tail muscle groups. Curly tail this extreme is seen only during stress such as the elevated beam walk shown here. (The beam is 21" off the surface, and the mice can slip off.) In the home cage, transient gentle curves are seen in the plane of the floor only. Note that this mouse has his legs under control and is walking the beam at the moment.

G. Mature mice show caudal hyperextension. In contrast, wild type mice spread their legs out in front.

H. Some young mice show claspings.

I. Extreme hyperextension, rigidity and very spread toes are seen during swim stress.

J. Crossing the beam by dragging very dystonic legs.

K. This is a screen shot of running taken in the dark with an infrared video camera.

The video showed rapid, coordinated running with occasional hindlimb slips.

The original practical aims of the project were to produce symptomatic dystonia in mice by mimicking the effects of a human genetic form of dystonia, Rapid-Onset Dystonia-Parkinsonism (RDP). Our collaborators identified the gene for one of the subunits of Na,K-

ATPase, ATP1A3, as the site of mutations in RDP (de Carvalho Aguiar et al. 2004). Our prior work in a knockout of the same gene in mice showed very little in the way of symptoms, and a surprising 80% recovery of both the Atp1a3 protein ($\alpha 3$) and activity. This we determined to be due to the continued full expression of the $\beta 1$ subunit, from the Atp1b1 gene. The hypothesis to be tested here was that reduction of the $\beta 1$ subunit by using a floxed Atp1b1 gene mouse in combination with reduction of the $\alpha 3$ subunit by breeding together with heterozygotes of the Atp1a3 knockout, would produce dystonia. Specifically, RDP is triggered by episodes of stress, and the mice were to be stressed by swimming or alcohol to mimic binge drinking, both typical of the stressors that trigger onset of symptoms in RDP.

That is all we anticipated being able to accomplish in one year with a \$75,000 budget, but once we had dystonic mice, we intended to obtain new funding to investigate the circuits that are hyperactivated during dystonia, with the aim of understanding the physiology well enough to work on a pharmacological treatment. Even though the original practical aims were not achieved, the important broader aim was achieved, and we are very excited about the prospects of putting the new mouse to work.

An ideal mouse would have dystonic symptoms without experimental interventions; no early mortality; ability to breed; high penetrance; no or slow progression; and high inter-individual consistency. The new mutation has the features desired in a mouse model. Notably, it exhibits kinesigenic dystonic hindlimb movements and non-kinesigenic postures, yet it can walk, and run on a wheel, consistent with a lack of neuropathy or spinal cord defects. It does not exhibit spasmodic episodes of generalized dystonia, or an ataxia syndrome, but more closely resembles segmental dystonias. Unlike mutant mice with multisystem effects, this mouse has normal initial weight gain, breeds well, and needs no special care. It has all the properties of an experimentally-useful animal model.

The significance of hindlimb symptoms.

Hindlimb clasping during tail suspension is often the first test for motor abnormality, as it is a rather low-threshold motor symptom seen in many mutant mice. Its neurological basis is not well understood. Hindlimbs have a dominant place in sensorimotor cerebellar integration in mice compared to primates, though, and hindlimb symptoms are also prominent in other rodent dystonia models, including surviving homozygotic Tor1A ΔE knock-in pups. An emerging hypothesis is that hindlimb and tail are a common locus of dystonia symptoms in the mouse.

Another hypothesis for hindlimb and tail selectivity is axonopathy affecting the longest axons most, but the data here so far, including histology and electrophysiology, do not support that. In sections examined by microscopy, peripheral nerve and spinal cord looked normal. The ability to run normally, despite persistent dystonic movements and postures, suggest that sensory as well as both upper and lower motor pathways are intact, as well as cerebellar pathways mediating the integration of ascending spinal inputs and vestibular inputs. Some pathways could, however, be overactive.

Spinal reflex circuits integrate sensory and proprioceptor information with motor input and relay that to the brain. Dystonia may be "posture gone overboard" or "error correction gone overboard": normal cerebellar and basal ganglia mechanisms for unconscious motor adjustments amplified to the point of impairment. If spinal circuits are intact in this mouse, functional defects may be found in sensorimotor integration, the basal ganglia, cerebellum or thalamus, such as "pausing" neurons in globus pallidus or defects in cerebellothalamocortical pathways, or firing alterations in striatal interneurons or Purkinje cells. If spinal circuits here are overactive, even if due to the modifier gene, that could be relevant to consider in the etiology of dystonia.

History and principal features.

The mouse arose in a colony carrying a knockout allele for an unrelated gene. The colony had been back-crossed to C57BL/6 every generation, and het x het matings produced WT, het, and KO littermates. The proband was WT for the knocked-out gene. Dominant inheritance was readily established. Both sexes breed. Weight gain is initially normal, but plateaus at a lower weight in adults (see Fig. 3 below). More than 100 affected mice, including the hybrids used for gene mapping, have been produced. Breeding efficiency is equal to other mice of this strain background, with 50:50 mutant and littermate controls. No affected mice have died without other unrelated cause. Onset is at 3-4 weeks, rarely later than that in C57Bl/6.

Behavior observations.

Figure 1 shows abnormal walking and sitting; tail suspension, in which caudal hyperextension predominates; beam test, showing adaptive behavior; swimming, showing hyperextension; near-normal voluntary running on a wheel in the dark; and hindlimb extension in apparent sleep. Hyperextension of the hindlimbs is the dominant symptom. Caudal hyperextension is seen when sleeping mice are awakened and take their first steps, but then the animals gain some control. During continued ambulation, the legs often show caudal or laterocaudal kicks, clearly kinesigenic. Both legs or one can be affected. At any given time, one may appear more impaired than the other, then switch. We hypothesize that this variable laterality reflects supra-spinal organization. When mice are fully alert, active and undisturbed, close-to-normal voluntary walking is seen with infrequent aberrant movements. They rear up readily on extended legs, and climb the air intake with effort. Disturbance or a novel environment elicits characteristic symptoms.

Sitting hindlimb posture is frequently abnormal. The angle between tibia/fibula and femur is considerably larger than normal, consistent with hyperextension. The feet are also often spread laterally, i.e. wide sitting stance. These are non-kinesigenic abnormal postures, and appear to be due to hyperextension, not ataxia.

The tail is held normally, but transiently adopts S-shaped curves caused by activation of ventrolateral tail segmental muscle groups. In the youngest, most active mice, a curvy tail is often visible even when they are otherwise moving normally. The forelimbs and head look normal. Occasional hunched posture resembling camptocormia is the only suggestion of axial involvement. Voluntary activities suggest a normal level of curiosity and exploratory behavior, and the affected mice voluntarily climb upside down on their food rack.

Affected mice display caudally extended hindlimb position during apparent sleep, scored ~50% of the time in young mice most of the time in older mice. Their sleep is very fidgety, and its neurological quality (REM vs. non-REM) is unknown.

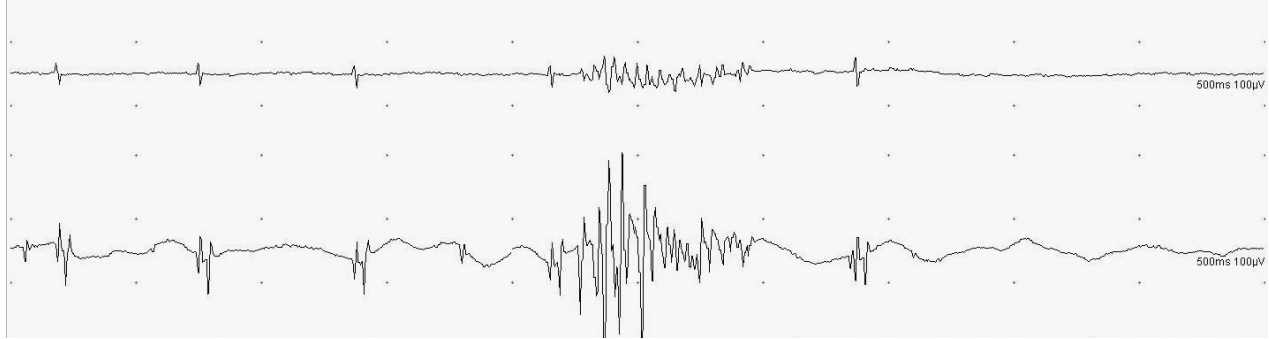
Many symptoms reported in other mutant mice are not seen: seizures, obviously ataxic gait, circling, hopping, creeping, unsteadiness, hyperkplexia, lethargy, or paralysis. No evidence of upper motor neuron syndrome or the cogwheel rigidity of parkinsonism was observed.

Neurological tests and electrophysiology.

The hindlimb site is reminiscent of axonopathies, yet the evidence argues against them. Stained sections of sciatic nerve looked completely normal. Quantitative assessments were made with cohorts of 18 mice: Stretch reflexes were felt when the hindlimb is relaxed, but suppressed when it is extended. Righting reflex was normal. The hot water tail flick test (nociceptor reflex) showed no difference in withdrawal time. Interestingly, stimulation of muscle spindles by vibration (with a hand-held battery-operated nail polisher tipped with tubing to create a focal contact point) elicited symptoms, as also seen in writer's cramp, a focal dystonia.

Electromyography (EMG) recordings were made with collaborator Dr. Johnny Salameh. Recordings of baseline and motor unit activity ruled out myotonia and spasticity, two conditions with genetic causes that could produce similar symptoms. Electrodes in opposing muscles showed co-contraction in all of the tested mice (Figure 2), a classic hallmark of dystonia. Electrodes in the same muscles of different hindlimbs, and in forelimb and hindlimb, detected no co-contraction, ruling out uncoordinated storms of activity. Nerve conduction velocity measures the integrity of large diameter myelinated axons, normal range 50-100 m/sec, and neuropathy would typically give readings of <30 m/sec. It was an average of 63 m/sec in studies of 2 and 4-month old affected and control mice. Amplitudes of compound motor action potentials were also

Fig. 2. Co-contraction of opposing muscles. 2-electrode EMG recordings



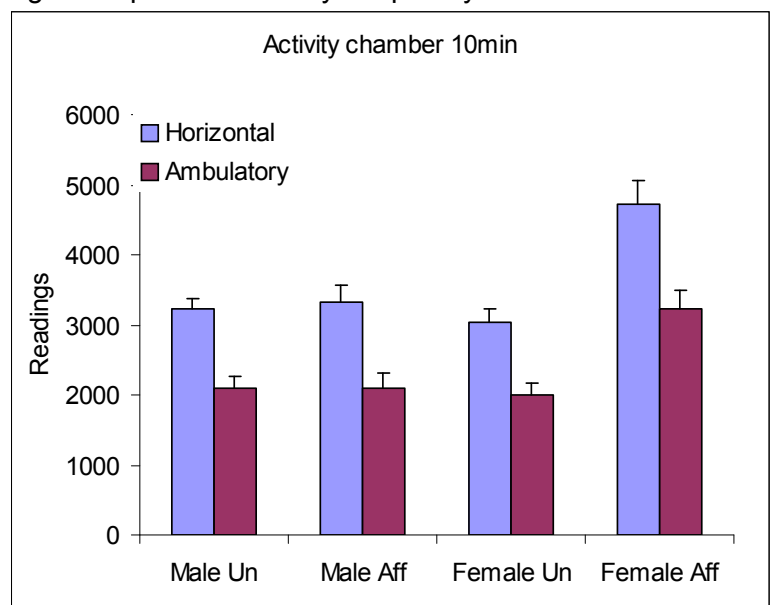
normal.

Motor tests.

It is characteristic of this mouse line to show quite dramatic symptoms, but then to gain some control: fall off a beam with rigid legs, then walk it, then show slips. Dystonia patients often have sensory tricks that allow them to similarly obtain temporary control. Tests were auditioned that would to some degree differentially assess cerebellar function (beam walk, pole climb and turn, Rotarod), peripheral nerve and limb strength (conduction velocity, nociceptor reflex, Rotarod and wire grip), and forelimb function (sticker swipe).

The battery of motor tests was completed with a cohort (n=18/group) of 3-5 month old C57BL/6 affected mice, unaffected mice, and WT controls. Activity field measurements found no statistically significant difference (Fig. 3). This is an important result because it shows how selective the mutation is for dystonia, in that it does not produce generalized lethargy or generalized hyperactivity, and it does not produce conditions mimicking anxiety that would cause the mice to stay at the edge.

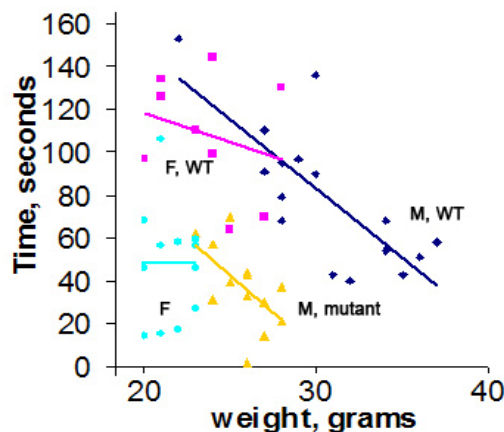
Fig. 3. Equivalent activity despite dystonia



Several other tests showed no statistical effect of genotype or high variability: Sticker swipe from the snout with the forelimbs was normal. In a wire rack grip test, scores were reduced in the mutant but the mice also had obvious adaptive behaviors, using feet like hooks and holding on with curvy tails. These adaptive behaviors confounded the use of the test for quantitative data. Beam walk showed impairment with variable results, since affected mice alternated among falling off, crossing with extremely dystonic posture, and walking across with some slips. At 4 weeks, all mice navigated the beam, but the affected ones had significantly more hindlimb slips. At 6 weeks, many affected mice navigated the beam but with continuous slipping, and some were unable to stay on. At 8-16 weeks, there was no further change.

During tail suspension, clasping was seen occasionally, but more commonly there was caudal (upward) hyperextension of the hindlimbs (Figure 1 G). To our knowledge this dystonic symptom is unique to this mouse.

Fig. 4. Rotarod motor test



Female (F) and male (M) mice scored for time on the accelerating Rotarod, plotted against their body weight. Mutants did much worse (aqua and yellow).

The accelerating Rotarod showed very significant differences superimposed on a main effect of weight (Fig. 4). The mutant mice were unable to use adaptive behaviors to remain on the rotating rod, and the test is stressful enough to maximize the dystonic response. This is suitable for future quantitative assessments, as in drug-testing. As can be seen from the data, weight matters a lot in the performance of even wild types once they are more than 25g.

In a pilot, three affected mice and matched littermates were tested on metered running wheels. They ran long distances and cumulative times (>4h per night). With this limited data, the affected mice appeared to run ~20% less. They were filmed running on wheels in the dark with an infrared video camera, and it is notable that they execute this activity well, which employs spinal locomotor central pattern generators.

The genes were successfully mapped

After crossing with FVB mice, DNA was used for SNP marker analysis with a medium-density Illumina linkage panel. DNA from the 10 most-affected N2-generation hybrids with FVB was submitted, and unambiguous loci comprising 20% of Chromosome 10 and 40% of Chromosome 12 were obtained. The genes in the loci were studied, and it ruled out the genes of all mouse strains with dystonic symptoms; all identified Dyt, Park, SCA and HSP genes; all GABA, glycine, glutamate, dopamine, cholinergic, opioid, adenosine, adrenergic, and 5HT receptors and related; and most ion channels and transporters. There were, however, a number of interesting candidate genes in the loci, including 3 K⁺ channels and 20 other genes that have knockout mice with neural effects; too many candidates for easy predictions.

Crossing also revealed a modifier gene that makes symptoms stronger in the C57BL/6 strain than in hybrids with FVB, DBA, and 129 strains. Symptoms that were unambiguous at 3-4 weeks in C57BL/6 mice took 5-9 weeks to emerge in F1 and N2 hybrids with FVB. That there is a second gene was verified definitively by the two separate loci found in the SNP marker analysis. This is an exciting development: one highly penetrant dominant mutation, and a modifier that produces no motor symptoms in C57BL/6 by itself. A Mendelian distribution (25% moderately-affected, 25% weakly-affected, 50% no symptoms) indicated that the modifier is homozygotic in B6 and has a dose-dependent effect. The existence of baseline genetic defects

in mice is well-known. All lab mice were *de facto* selected to be slow and tame, and inbreeding made such traits homozygotic. For example, C3H and several others have a loss-of-function Parkin missense mutation. Blindness and deafness mutations are carried by many strains, a reason that C57Bl/6, which is not blind or deaf, is a strain of choice for neuroscience. Strain-specific modifier effects on longevity were found in Dyt1 model mice. D1 and D2 receptor effects also differed among strains, with implications for striatal function. In this context, the discovery of a modifier gene affecting manifestation of dystonia is very significant.

Fig. 5 shows an example of the SNP mapping results for chromosome 10; similar results were obtained for the 2nd locus on chromosome 12. Only regions in which every one of the 10 affected mice has the same genotype (yellow) qualifies as a locus.

Fig. 5. SNP mapping results. Not all SNPs are different between the two mouse strains; only the ones highlighted yellow are informative. DNA from 10 different mice was submitted. Blue boxes show informative recombination events. The deduced region of interest is highlighted in blue on the right.

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10	119351908	CEL-10_119602638	AB	AB	AB	AB	AB	AB	AB	AB	AB	BB	BB	BB	AA	AA
10	122568425	rs13480803	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
10	125735679	mCV22832306	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
10	126250086	rs3697243	AB	AB	AB	AB	BB	AB	AB	AB	AB	BB	BB	BB	AA	AA
10	127847104	rs3676330	AB	AB	AA	AB	AA	AB	AB	AB	AB	AB	AA	AA	BB	BB

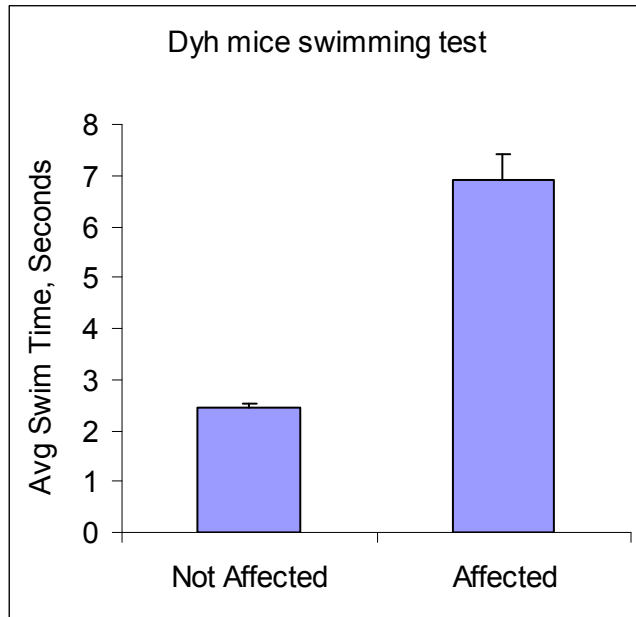
Chr 10

ROI

3. 3rd 6 months, the no-cost extension

Using large groups of successfully bred mice, we extended the behavior tests to identify a second major quantitative test that will be useful for systematic drug testing. (As reported

Fig. 6. Swim speed test.



above, some common measures were similar to wild type, and some others proved not-suitable because the mice learn adaptive behaviors that allow them to perform variably well despite obvious motor abnormalities.) The accelerating rotarod test (Fig. 4 above) was very successful, and a second test was developed for independent information.

Swimming speed down a lane to a platform showed statistically very significant >2-fold differences (Fig. 6). This was done in a custom-constructed swimming pool.

Fig. 7 shows that there was no main effect of age on swimming speed, and Fig. 8 shows that there was a small main effect of weight but only for mutant males, which are of course larger.

Fig. 7

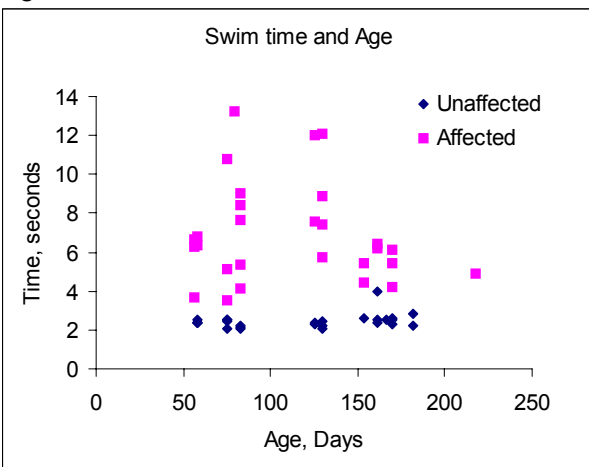
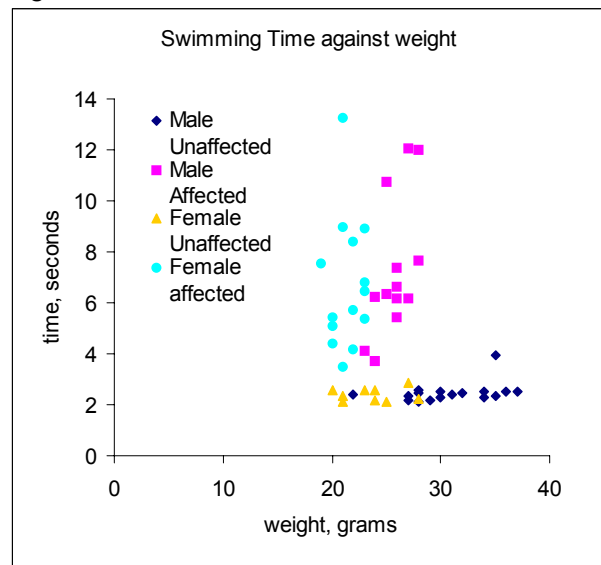


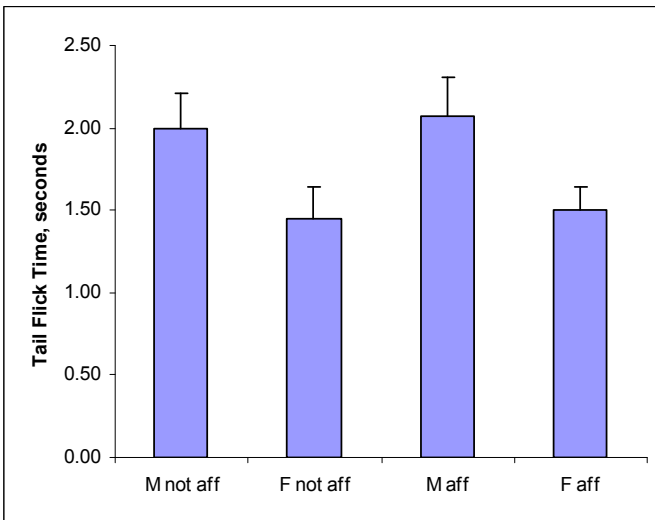
Fig. 8



This in depth analysis supports the use of the mice for drug testing for treatments for dystonia.

A final behavioral test completed was the tail flick test. The tip of the mouse's tail is immersed in hot water at a temperature of 50 °C, and the time it takes for it to flick the tail up and out of the water is timed. This is a measure of the speed of conduction of sensory nerves with the speed of spinal reflex pathways back to the tail musculature. The lack of an effect of the mutation reinforces the finding of normal histology in spinal cord and sciatic nerve, ruling out neuropathy. (Fig. 9) There is a sex difference that reaches significance.

Fig. 9. Tail flick test of sensory nerve conduction velocity



Finally, we completed another generation of hybrid back-crossing for the purpose of further reducing the size of the two genetic loci that were identified. At this point we have more potentially informative DNA samples than we have funds to complete the SNP analysis, and we are hoping to continue the work with a grant from the NIH.

The last experiment, which is currently awaiting final DNA results, is to use SNP mapping to determine which locus is the main, mutant gene, and which is the modifier gene. To this end we used exhaustive testing of the hybrid mice to separate them into "Moderately affected" (one copy of the main gene and one copy of the modifier gene), "Weakly affected" (one copy of the main gene only), and "Unaffected" (wild type at the main gene locus and either one or no copy of the modifier gene). Ratios: 24:26:50. We used the following screens:

1. Repeated cage observation of abnormal movement.
2. Vibration-induced dystonia.
3. Abnormal hindlimb movement during tail suspension.
4. Dystonic activity during light isoflurane anesthesia.
5. Dystonic activity during recovery from isoflurane anesthesia
6. Dystonic activity during sleep.
7. Dystonic activity during swimming.

We have now submitted 8 "weak" mice for SNP mapping, and expect definitive results within two weeks.

We are still awaiting the planned whole exome sequencing by the Broad Institute, which should identify the main mutant gene. Although our project was approved last August and DNA samples were accepted by the Broad in January, they only recently accumulated enough other mouse samples to complete the run. We eagerly await the results, and plan to publish everything in a single definitive paper.

Key research accomplishments

- We have completed basic characterization of the first mouse with apparently pure dystonia symptoms, without baseline deficiencies, and easy to breed.
- We have assessed swim-stress as a trigger for dystonic symptoms.
- We have used EMG recordings to show that there is co-contraction of opposing muscle groups, the hallmark of dystonia.
- We have used SNP mapping to determine not one, but two genetic loci, one for the main dominantly-inherited gene, and the other for a modifier gene that controls manifestation.
- We have established two different behavioral tests that are quantitative and suitable for using from drug testing in this mutant mouse.
- We are determining which locus is the main gene and which the modifier gene.
- We have submitted samples for Next Generation (whole exome) sequencing.

Reportable outcomes

1. The mouse was presented at the international Movement Disorders Society meeting in Dublin, June 17-21, 2012:

Sweadner, KJ, Liu, YB, Ozelius, LJ, Brashear, A. 2012. A new mutant mouse with symptoms of dystonia. *Movement Disorders* 27 Suppl 1: S367.

2. It was also presented at the ENRAH (European Network for Research on Alternating Hemiplegia) meeting in Belgium, December 11-12, 2012. (30 minute talk was given).

Funding has been applied for from the following sources:

Dystonia Medical Research Foundation

Bachmann-Straus Dystonia and Parkinson Foundation

Harvard NeuroDiscovery Research Center

Massachusetts Neuroscience Consortium

NIH, NINDS. An application for an R21 has been scored in the 10th percentile, and awaits action by Council. Total direct costs: \$275,000.

Conclusion

The original aims of the grant are still of significant scientific interest for understanding how RDP and its juvenile form, AHC, bring about dystonia, and we now have a mouse with mutated Na,K-ATPase with AHC symptoms to use for future pharmacological efforts to produce a treatment. However, the new mouse line described here will be just as valuable as a practical research tool, and we hope to identify the dominant mutated gene by July, the Broad Institute's schedule. Because the new gene will be novel, it may lead to the discovery of corresponding mutations in human inherited dystonias where a gene has not yet been identified. Most importantly, the new mouse has stress-induced symptoms, and so it will be a good animal model for future investigation of the circuits and electrophysiology that underlie stress effects on movement disorders. That in turn is highly relevant to finding treatments.

References

Sweadner, KJ, Liu, YB, Ozelius, LJ, Brashear, A. 2012. A new mutant mouse with symptoms of dystonia. *Movement Disorders* 27 Suppl 1: S367.

Appendices and supporting data

The abstract published in *Movement Disorders* is included.

additional therapies. Other questions were related to patient awareness, frustration to reach the diagnosis, lack of general understanding of the disorder, and willingness to actively participate in increasing awareness about dystonia. 114 patients responded and the results were analyzed.

Results: Patients visited a median of 3 doctors, and 34.5% saw 5 or more physicians before receiving a correct diagnosis of dystonia. It took more than 1 year for 57% of patients to have a correct diagnosis of dystonia. For 38% of participants it took 2 or more years to decide to visit a doctor for their symptoms. The majority of patients were correctly diagnosed by a neurologist (72%). 57% were originally misdiagnosed and 10% of those were told that they were making up the symptoms.

Conclusions: Dystonia was difficult to diagnose properly in 1/3 of patients, requiring to visit multiple physicians over an extensive period of time. Over half of the patients were initially misdiagnosed, taking 2-4 years to have a correct diagnosis. There is poor awareness about dystonia outside neurologists. Patients think that there is lack of knowledge in the community about dystonia, but a minority are willing to participate in efforts to increase awareness of dystonia.

1110

A new mutant mouse with symptoms of dystonia

K.J. Sweadner, Y.B. Liu, L.J. Ozelius, A. Brashear (Boston, MA, USA)

Objective: To obtain a mouse model that exhibits consistent symptoms of dystonia for future pathway analysis and testing of drugs and interventions.

Background: While there are many mutant mice with measurable motor deficits, it has been difficult to obtain mice with obvious symptoms of dystonia. Some display paroxysmal dystonia only when treated with drugs; others have severe neuropathological features or are difficult to keep alive. A useful mouse would have reliable symptoms, but require no special care. Spontaneous mutations of more than 20 genes cause dystonia in humans.

Methods: A new mutation arose spontaneously in a colony of C57BL/6 mice. Genetic inheritance was established by breeding with a sibling, then by additional crosses to affected offspring and to wild type mice. Out-crossing and marker analysis to determine the genetic locus is underway. Dystonic movements and postures were documented with video in pilot motor behavioral studies.

Results: The new mutant proband was the only affected mouse out of 27 siblings, but when mated, produced 6 affected offspring out of 13 pups so far, suggesting de novo occurrence and dominant inheritance. All affected mice have identical hindlimb symptoms that resemble posturing types of dystonia. Excessive contraction of extensors results in caudal and lateral hyperextension that is seen upon awakening and moving around the cage. Hindlimb hyperextension continues during sitting, and the feet jut forward and laterally. However, the mice can also walk well, albeit sometimes with hindlegs and pelvis elevated by hyperextension. Most notably, they can run quite well on a wheel, running for hours a night and at ~80% of the speed of unaffected littermates in preliminary experiments. They maintain weight; groom well, breed readily; show exploratory behavior; and climb voluntarily despite hindlimb problems.



Fig. 1 (1110).

Conclusions: Importantly, the ability to execute wheel-running suggests that the mice have intact upper motor neurons, spinal pattern generators, and segmental motor and sensory innervation. We propose that the primary impairment is supra-spinal. The mouse's ability to override dystonic postures to walk and run may be analogous to sensory tricks in human dystonia. This mouse merits study of its genetics, neuropathology, and physiology to determine if it is a useful model of dystonia.

1111

Dopa-responsive dystonia revisited: Diagnostic delay, residual signs, and non-motor signs

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Objective: To revisit delay in diagnosis (DID), residual signs (RS) and non-motor signs (NMS) of Dopa-responsive dystonia (DRD) using literature and own pilot data.

Background: DRD is caused by an autosomal dominant mutation in the GCH1 gene with incomplete penetrance. Despite its pathognomonic clinical features, DRD is often undiagnosed or with considerable delay and little is known about RS and biologically plausible, serotonin-related NMS.

Methods: We searched the Medline database for patients with clinically typical DRD and/or GCH1 mutations from the first description to 2011, and examined 23 DRD outpatients with proven GCH1 mutations.

Results: The literature search yielded 101 reports describing 576 DRD cases. After excluding cases without a proven GCH1 mutation, homozygous and asymptomatic mutation carriers, 352 cases were evaluated. The pilot study comprised 12 index patients and 11 affected family members. In cases with available information, age at onset (AAO) was 11.6 ± 13.4 years (literature) and 12.9 ± 20.2 years (pilot study) and the DID was 15.2 ± 13.7 years (literature) and 15.1 ± 16.5 years (pilot study). RS on therapy were reported in 28% (literature) and found in 48% (pilot study) of the patients. RS in the literature included dystonic (20%) and parkinsonian (11%) symptoms, and complications (9%) such as contractures. In 12 cases, patients had undergone unnecessary surgical procedures. Information on NMS was given for 70 cases, including depression (34%), anxiety (19%), and obsessive-compulsive disorders (9%). Eight of our own cases (35%) reported one or more NMS.

Conclusions: Despite the well-known etiology of DRD, availability of genetic testing and specific therapy, DID is considerable. In addition, a sizable number of treated patients with DRD have RS, including motor signs, NMS and complications resulting from lack of timely therapy or unnecessary procedures.

1112

Distribution of mutant torsinA in living cells

I. Toyoshima, E. Abe, S. Kamada (Yurihonjo, Japan)

Objective: To analyze the distribution of wild type and mutant torsinA in living cells we employed new fluorescent fusion proteins.

Background: Mutation of torsinA is responsible for DYT1 dystonia. TorsinA is one of the AAA+ proteins and is thought to result in synaptic pathology. Membrane distortion derived from mutant torsinA thought to cause disturbance of membrane trafficking and poverty of synaptic vesicles.

Methods: We newly cloned wild type torsinA from cDNA library of human brain and obtained GAG deleted (deIE) clone by mutagenesis. AcGFP and DsRed-monomer (Clontech) were used for production of fusion proteins with torsinA. Golgi apparatus and endoplasmic reticulum (ER) were visualized by fluorescent proteins simultaneously. All were transfected into Cos7 cells and SHSY5Y cells.

Results: TorsinA-AcGFP both wild type and deIE mutant localized perinuclear region with small vesicular structure in Cos7 cells. Nuclear membrane was faintly labeled. Vesicular structure colocalized with Golgi apparatus derived membrane but segregated with ER luminal protein. On the other hand, torsinA-DsRed-monomer distrib-